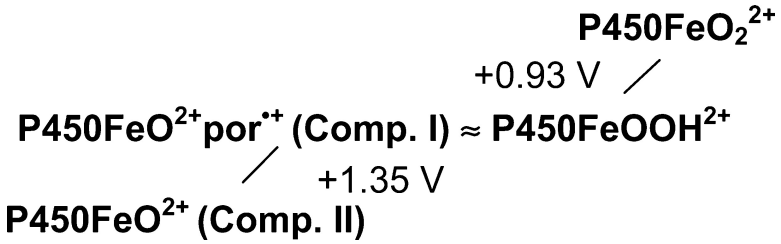
Article

Oxygen Activation by Cytochrome P450: A Thermodynamic Analysis

Willem H. Koppenol

J. Am. Chem. Soc., **2007**, 129 (31), 9686-9690• DOI: 10.1021/ja071546p • Publication Date (Web): 13 July 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 5 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 07/13/2007

Oxygen Activation by Cytochrome P450: A Thermodynamic Analysis

Willem H. Koppenol

Contribution from the Institute of Inorganic Chemistry, Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

Received March 5, 2007; E-mail: koppenol@inorg.chem.ethz.ch

Abstract: Electrode potentials for every intermediate in the cytochrome P450 cycle were estimated and evaluated by means of an oxidation state diagram. By this approach, and within the uncertainties of the approximations, the superoxide complex of cytochrome P450 at pH 7 is oxidizing: $E^{\circ\prime}$ ($P450FeO_2^{2+}$, $H^+/P450FeOOH^{2+}$) = +0.93 V, and the Gibbs energy for the reaction of the hydroperoxo complex of cytochrome P450 to form compound I and water, $P450FeOOH^{2+} + H^+ = P450FeO^{2+}por^{\bullet+} + H_2O$, is 0 kJ/mol. Although cytochrome $P450FeOOH^{2+}$ and cytochrome $P450FeO^{2+}por^{\bullet+}$ are approximately isoenergetic, they are likely to react at different rates with substrates and may yield different products. Homolysis of the hydroperoxo complex of cytochrome P450 to compound II and the hydroxyl radical, $P450FeOOH^{2+} = P450FeO^{2+} + HO^{\bullet}$, is unfavorable ($\Delta G^{\circ\prime}$ = +92 kJ/mol), as is the dissociation into HOO^- and cytochrome $P450Fe^{3+}$ (+73 kJ/mol). It is shown that the sum of the Gibbs energy of association for cytochrome $P450Fe^{3+}$ with the hydroperoxo anion and the Gibbs energy for the one-electron reduction of cytochrome $P450FeOOH^{2+}$, relative to NHE, is constant (−203 kJ/mol). While the estimated $E^{\circ\prime}$ ($P450FeO_2^{2+}$, $H^+/P450FeOOH^{2+}$) = +0.93 V at pH 7 is larger than necessary to effect reduction of cytochrome $P450FeO_2^{2+}$, the magnitude of this electrode potential implies that the binding constant for cytochrome $P450Fe^{3+}$ with hydrogen peroxide is ca. 3×10^6 M^{-1} at pH 7. An association constant of this magnitude ensures that a fraction of cytochrome $P450FeOOH^{2+}$ is available to form compound I or to react with substrates directly, while a larger one would imply that compound I is too weak an oxidant. In general, the energetics of the reduction of dioxygen to water determines the energetics of catalysis of hydroxylations by cytochrome P450. These results enable calibration of energy levels obtained for intermediates in the cytochome P450 reaction cycle obtained by ab initio calculations and provide insights into the catalytic efficiency of cytochrome P450 and guidelines for the development of competent hydroxylation catalysts.

Introduction

Cytochrome P450 carries out hydroxylations as well as epoxidations and decarboxylations through the activation of dioxygen for the insertion of an oxygen atom into a substrate.[1−3] This process involves the reduction of dioxygen to hydrogen peroxide and the subsequent formation of higher oxidation states of iron capable of transferring oxygen. The hydroxylating agent in the cytochrome P450 cycle may be (i) the equivalent of compound I of the peroxidases, in which the heme iron, formally in the iron(IV) state, bears an activated oxygen with an oxidizing equivalent on the porphyrin ring ($P450FeO^{2+}por^{\bullet+}$), (ii) heme iron(III) bound to a hydroperoxide ($P450FeOOH^{2+}$), or (iii) oxidoiron heme with iron in the 5+ state ($P450Fe^{V}O$). These intermediates are short-lived and, thus, difficult to characterize, which thereby hampers the understanding of the energetics of the catalysis. Here, electrode potentials for every intermediate in the cytochrome P450 cycle are estimated and evaluated by means of an oxidation state diagram.

The overall hydroxylation reaction can be represented as a half-reaction (eq 1)

$$RH + O_2 + 2H^+ + 2e^- = ROH + H_2O \qquad (1)$$

with $E^{\circ\prime} \cong +1.5$ V at pH 7, based on the electrode potentials for the CH_3OH, H^+/CH_4, H_2O, and O_2, H^+/H_2O couples.[4,5]

About a decade ago, observations of different reactivities led to the proposal that, in addition to the accepted intermediate cytochrome $P450FeO^{2+}por^{\bullet+}$,[6] another oxidant, the iron(III) hydrogen peroxide adduct (cytochrome $P450FeH_2O_2^{3+}$ or its deprotonated form), is active during the catalytic cycle of cytochrome P450.[7,8] However, others have ascribed these reactivities to two different spin states of the oxidant cytochrome

(1) Guengerich, F. P.; MacDonald, T. L. *FASEB J.* **1990**, *4*, 2453−2459.
(2) Denisov, I. G.; Makris, T. M.; Sligar, S. G.; Schlichting, I. *Chem. Rev.* **2005**, *105*, 2253−2278.
(3) McCarthy, M.-B.; White, R. E. *J. Biol. Chem.* **1983**, *258*, 9153−9158.
(4) Koppenol, W. H.; Rush, J. D. *J. Phys. Chem.* **1987**, *91*, 4429−4430.
(5) Hoare, J. P. Oxygen; In *Standard Potentials in Aqueous Solution*; Bard, A. J., Parsons, R., Jordan, J., Eds.; Marcel Dekker, Inc.: New York, 1985; pp 49−66.
(6) Kim, S. H.; Perera, R.; Hager, L. P.; Dawson, J. H.; Hoffman, B. M. *J. Am. Chem. Soc.* **2006**, *128*, 5598−5599.

10.1021/ja071546p CCC: $37.00

$P450FeO^{2+}por^{\bullet+}$.[98] This controversy has been the subject of a forum.[10−12] Recently, it has been proposed that the hydrogen peroxide adduct initially forms a true oxidoiron(V) intermediate, which would subsequently decay to form the less reactive oxidoiron(IV) porphyrin radical cation or compound I.[13] Although the dioxygen adduct of iron(II) cytochrome P450 has been isolated[14] and even crystallized,[15] the hydroperoxide adduct[16] has been observed only when frozen samples are exposed to ionizing radiation (see Denisov et al.[2]). The compound I and II states of cytochrome P450 are, unlike those of peroxidases, similarly elusive. Only recently, it was reported that the reaction of cytochrome P450 with peroxynitrite yielded compound II and that photo-oxidation of the latter produced compound I.[17] This analysis deals with the thermodynamic properties of putative intermediates commonly described in the literature; it was not attempted to ascribe the different reactivities that have been observed to any of these intermediates.

The importance of the spin state of iron(III) cytochrome P450 and the presence or absence of a substrate with respect to the energetics of reduction and dioxygen binding have been discussed in the literature.[18,19] What is presented here is the first complete description of the thermodynamics of the cytochrome P450 cycle. To perform the thermodynamic analysis, it was necessary to make three assumptions, but the resulting uncertainties in the thermodynamic values derived are relative, and the overall conclusions are not influenced by these assumptions. For example, some values are derived by assuming that the oxidizing species of cytochrome P450 are similar to those of myeloperoxidase. In general, the results provide boundary conditions for certain values, such as dissociation and homolysis energies, generated by ab initio calculations, and the electrode potentials offer insight into the oxidizing nature of the dioxygen-iron(II) (compound III) and peroxo-iron(III) intermediates.

Calculations and Discussion

All electrode potentials and Gibbs energies refer to pH 7. For the electrode potential of the iron(III)/iron(II) couple of cytochrome P450, a value that was determined with substrate present, −0.17 mV,[18] was used; however, it should be noted that values more negative by ca. 0.2 V also have been reported.[2,20] The dissociation constant for the dioxygen complex is 1.4×10^{-6} M,[21] equivalent to a Gibbs binding energy of −17.0 kJ/mol (pO_2 = 100 kPa). Thus, the Gibbs energy of reaction 2 (relative to the normal hydrogen electrode) is −0.6 kJ/mol.

Table 1. Electrode Potentials

half-reaction	$E^{\circ\prime}$ (vs NHE, pO_2 = 100 kPa) at pH 7 (V)
$O_2(g) + e^- = O_2^-$	−0.35[a]
$O_2^- + e^- + 2H^+ = H_2O_2$	+0.91[a]
$O_2(g) + 2e^- + 2H^+ = H_2O_2$	+0.28[a]
$H_2O_2 + 2e^- + 2H^+ = 2H_2O$	+1.35[a]
$O_2(g) + 4e^- + 4H^+ = H_2O$	+0.81[a]
$HO + e^- + H^+ = H_2O$	+2.31[a]
$H_2O_2 + e^- + H^+ = HO + H_2O$	+0.39[a]
$P450Fe^{3+} + e^- + O_2 = P450FeO_2^{2+}$	+0.01[b]
$P450FeO_2^{2+} + e^- + H^+ = P450FeOOH^{2+}$	+0.93[b] (+1.04)[c]
$P450FeOOH^{2+} + e^- + H^+ = P450FeO^{2+} + H_2O$	+1.35[b]
$P450FeO^{2+} + e^- + 2H^+ = P450Fe^{3+} + H_2O$	+0.97[b]
$P450Fe^{V}O^{3+c} + e^- = P450FeO^{2+}$	+1.4[b]
$P450FeO^{2+}por^+ + e^- = P450FeO^{2+}$	+1.35[b]

[a] Values from an IUPAC evaluation of literature values (D. A. Armstrong, R. H. Huie, W. H. Koppenol, S. Lymar, G. Merényi, P. Neta, D. M. Stanbury, S. Steenken, P. Wardman, manuscript in preparation). [b] This paper; these values are estimates and have uncertainties of 0.1 V. [c] Adjusted to 0.93 V, see text. [d] $P450Fe^{V}O^{3+}$ is an abbreviation for oxidoiron(V) cytochrome P450.

$$P450Fe^{3+} + e^- + O_2 = P450FeO_2^{2+} \quad E^{\circ\prime} = +0.006\ V \tag{2}$$

The reverse of reaction 2, combined with reaction 3

$$O_2 + e^- = O_2^{\bullet-} \quad E^\circ = -0.35\ V \tag{3}$$

(Table 1) yields a Gibbs energy of +34 kJ/mol for the dissociation of superoxide from $P450FeO_2^{2+}$ (reaction 4), which is needed later.

$$P450FeO_2^{2+} = P450Fe^{3+} + O_2^{\bullet-} \tag{4}$$

The one-electron reduction potential of the dioxygen-Fe(II) or superoxide-Fe(III)/hydroperoxo-Fe(III) couple is calculated with the help of the following considerations. Given the pK_a of 11.6 for hydrogen peroxide, 26 kJ is required for deprotonation.

$$P450FeO_2^{2+} + H^+ + e^- = P450Fe^{3+} + HO_2^- \quad E^{\circ\prime} = +0.28\ V \tag{5}$$

$$P450Fe^{3+} + HOO^- = P450FeOOH^{2+} \quad E^{\circ\prime} = +0.76\ V \tag{6}$$

$$P450FeO_2^{2+} + H^+ + e^- = P450FeOOH^{2+} \quad E^{\circ\prime} = +1.04\ V \tag{7}$$

Reaction 5 is the sum of the dissociation of the dioxygen complex into cytochrome $P450Fe^{2+}$ and superoxide (reaction 4) and the reduction of superoxide to HO_2^- at pH 7 [pK_a (H_2O_2) = 11.6; thus, $E^{\circ\prime}$ ($O_2^{\bullet-}$, H^+/HO_2^-) = +0.64 V]. For reaction 6, the assumption is made that the dissociation energies of $P450FeOOH^{2+}$ (reaction −6) and $P450FeO_2^{2+}$ (reaction 4) are proportional to the dissociation energies of HOOH (pK_a = 11.6) and $HO_2^{\bullet}$ (pK_a = 4.8),[22] respectively. Given the +34 kJ/mol for reaction 4, this leads to −73 kJ/mol for reaction 6

(7) Pratt, J. M.; Ridd, T. I.; King, L. J. *J. Chem. Soc., Chem. Commun.* **1995**, *1995*, 2297−2298.
(8) Toy, P. H.; Newcomb, M.; Coon, M. J.; Vaz, A. D. N. *J. Am. Chem. Soc.* **1998**, *120*, 9718−9719.
(9) Shaik, S.; Filatov, M.; Schröder, D.; Schwarz, H. *Chem.−Eur. J.* **1998**, *4*, 193−199.
(10) Jin, S.; Bryson, T. A.; Dawson, J. H. *J. Biol. Inorg. Chem.* **2004**, *9*, 644−653.
(11) Nam, W.; Ryu, Y. O.; Song, W. J. *J. Biol. Inorg. Chem.* **2004**, *9*, 654−660.
(12) Shaik, S.; De Visser, S. P.; Kumar, D. *J. Biol. Inorg. Chem.* **2004**, *9*, 661−668.
(13) Harischandra, D. N.; Zhang, R.; Newcomb, M. *J. Am. Chem. Soc.* **2005**, *127*, 13776−13777.
(14) Debey, P.; Balny, C.; Douzou, P. *FEBS Lett.* **1976**, *69*, 231−235.
(15) Nagano, S.; Poulos, T. L. *J. Biol. Chem.* **2005**, *280*, 31659−31663.
(16) Davydov, R.; MacDonald, I. D. G.; Makris, T. M.; Sligar, S. G.; Hoffman, B. M. *J. Am. Chem. Soc.* **1999**, *121*, 10654−10655.
(17) Newcomb, M.; Zhang, R.; Chandrasena, R. E. P.; Halgrimson, J. A.; Horner, J. H.; Makris, T. M.; Sligar, S. G. *J. Am. Chem. Soc.* **2006**, *128*, 4580−4581.
(18) Sligar, S. G. *Biochemistry* **1976**, *15*, 5399−5406.
(19) Honeychurch, M. J.; Hill, H. A. O.; Wong, L.-L. *FEBS Lett.* **1999**, *451*, 351−353.
(20) Clark, J. P.; Miles, C. S.; Mowat, C. G.; Walkinshaw, M. D.; Reid, G. A.; Daff, S. N.; Chapman, S. K. *J. Inorg. Biochem.* **2006**, *100*, 1075−1090.
(21) Peterson, J. A.; Ishimura, Y.; Griffin, B. W. *Arch. Biochem. Biophys.* **1972**, *149*, 197−208.
(22) Bielski, B. H. J.; Cabelli, D. E.; Arudi, R. L. *J. Phys. Chem. Ref. Data* **1985**, *14*, 1041−1100.

Table 2. Gibbs Energy Changes[a]

reaction	$\Delta G^{\circ\prime}$, at pH 7 (kJ/mol)
$P450Fe^{3+} + O_2^- = P450FeO_2^{2+}$	−34
$P450Fe^{3+} + HOO^- = P450FeOOH^{2+}$	−73
$P450FeOOH^{2+} + H^+ = P450FeO^{2+}por^+ + H_2O$	0
$P450FeOOH^{2+} = P450FeO^{2+} + HO$	+92
$P450FeO^{2+} + H^+ = P450Fe^{3+} + HO$	+129

[a] Uncertainty in these estimates is ca. 10 kJ/mol.

(assumption 1, Gibbs energies are collected in Table 2.) The Gibbs energy change of reaction 8

$$P450Fe^{3+} + H_2O_2 = P450FeOOH^{2+} + H^+ \qquad (8)$$

is, then, −47 kJ/mol, which corresponds to a binding constant of 1.7×10^8 M^{-1} at pH 7. At micromolar concentrations of protein and hydrogen peroxide, nearly no hydrogen would dissociate, which is contrary to observations with cytochrome $P450_{cam}$ and non-camphor substrates.[23] The estimated binding constant of 1.7×10^8 M^{-1} at pH 7 is likely to be too high. The formation of compound I via oxidoiron(V) cytochrome P450, $P450Fe^{V}O^{3+}$, or reaction with substrate requires protonation of the distal $P450FeOOH_2^{3+}$ oxygen in $P450FeOOH^{2+}$, while protonation of the proximal oxygen is likely to lead to dissociation of hydrogen peroxide. Given that both dissociation of hydrogen peroxide and formation of compound I occur, the equilibrium for distal versus proximal protonation is on the order of 1. The pK_a of the coordinated hydrogen peroxide is not known but has been estimated to be 2−5 units below that of coordinated water.[24] The pK_a of water coordinated to iron(III) of cytochrome P450 is larger than 8.6[25] and may be close to 9, as determined for water bound to iron(III) in myoglobin.[26] Thus, the pK_a of cytochrome $P450FeHOOH^{3+}$ is approximately between 4 and 7 as is that of cytochrome $P450FeOOH_2^{3+}$. If the pK_a were as low as 4, cytochrome $P450FeOOH^{2+}$ would be expected to be rather stable and, therefore, observable, which it is not; thus, a value between 5.5 and 7 for the pK_a seems to be more reasonable (assumption 2). Given the pH 7 standard state, protonation requires only 0−9 kJ/mol.

The electrode potentials of the compound II/iron(III) and compound I/compound II couples of some peroxidases have been reported;[27,28] those for myeloperoxidase (MPO-I and -II) are shown here[27]

$$MPO\text{-}II + e^- + 2H^+ = MPO\text{-}Fe^{3+} + H_2O \quad E^{\circ\prime} = +0.97\ V \qquad (9a)$$

$$MPO\text{-}I + e^- = MPO\text{-}II \quad E^\circ = +1.35\ V \qquad (10a)$$

Interestingly, this experimentally determined electrode potential for reaction 9a is similar to that of +0.99 V, which was estimated by methods similar to those used in the present work.[29]

These reactions may be rewritten for cytochrome P450

$$P450FeO^{2+} + e^- + 2H^+ = P450Fe^{3+} + H_2O \quad E^{\circ\prime} = +0.97\ V \qquad (9b)$$

$$P450FeO^{2+}por^{\bullet+} + e^- = P450FeO^{2+} \quad E^\circ = +1.35\ V \qquad (10b)$$

Given that myeloperoxidase is known carry out some hydroxylations[30,31] and that the compound I and II states of cytochrome P450 are likely to be more reactive than those of myeloperoxidase, these electrode potentials may be lower limits for the P450 potentials (assumption 3). As a consequence of the protonation of $P450FeOOH^{2+}$, the electrode potential of the couple $P450FeH_2O_2^{3+}/P450FeO^{2+}$, H_2O would be ca. 1.4 V. If $P450FeH_2O_2^{3+}$ first decays to oxidoiron(V) cytochrome P450, then 1.4 V serves as an upper limit for the couple cytochrome $P450Fe^{V}O^{3+}$/cytochrome $P450FeO^{2+}$.

These results are presented in a Frost[32] or oxidation state diagram (Figure 1) for dioxygen, where $nE^{\circ\prime}$ is plotted versus n, the oxidation state of elemental oxygen. The slope of each line that joins two points, which represent a thermodynamic couple, gives the corresponding electrode potential. $nE^{\circ\prime}$ is proportional to the Gibbs energy per mol of oxygen. Thus, in Figure 1, on the right-hand axis, Fe^{3+}, O_2 is found at the coordinates 0, 0, and on the lower left axis, Fe^{3+}, H_2O is at $n = -2$ and $nE^{\circ\prime} = -1.63$. In the figure, cytochrome $P450Fe^{3+}$ is codified by Fe^{3+}. The location of the point Fe^{3+}, O_2 at 0, 0 implies that the Gibbs energy of formation of cytochrome $P450Fe^{3+}$ in water is arbitrarily set at 0 kJ/mol. Reduction of the iron(III) of cytochrome P450 and subsequent binding of dioxygen are represented by the red line from Fe^{3+}, O_2 to FeO_2^{2+} ($n = -1/2$) with a slope of +0.17 V. The iron(II)-dioxygen complex is oxidizing, with an electrode potential of +0.93 V. From the left to the middle, the electrode potentials for the couples compound II/Fe^{3+}, 0.98 V and compound I/II, 1.35 V of myeloperoxidase are shown in green. Although at, $n = -1$ the location of cytochrome $P450FeOOH^{2+}$ is slightly below that of myeloperoxidase Compound I and water, these compounds occupy, within the error of the assumptions, the same energy level. Thus, the Gibbs energy change for reaction 11 is very close to 0 at pH 7. The activation energy for this reaction, for which an estimate of ca. 60 kJ/mol has been published, is thought to be due to electronic reorganization.[33] Interestingly, according to the same ab initio (QM/MM) study,[33] the conversion of $P450FeOOH^{2+}$ to $P450FeO^{2+}por^{\bullet+}$ and H_2O with Asp 251 as the proton donor is also energetically neutral.

$$P450FeOOH^{2+} + H^+ = P450FeO^{2+}por^{\bullet+} + H_2O \qquad (11)$$

It should be re-emphasized that cytochrome P450 is not myeloperoxidase: the compound I equivalent of cytochrome

(23) Zhang, Z.; Sibbesen, O.; Johnson, R. A.; Ortiz de Montellano, P. R. *Bioorg. Med. Chem. Lett.* **1998**, *6*, 1501−1508.
(24) Mirza, S. A.; Bocquet, B.; Robyr, C.; Thomi, S.; Williams, A. F. *Inorg. Chem.* **1996**, *35*, 1332−1337.
(25) Philson, S. B.; Debrunner, P. G.; Schmidt, P. G.; Gunsalus, I. C. *J. Biol. Chem.* **1979**, *254*, 10173−10179.
(26) George, P.; Hanania, G. *Biochem. J.* **1952**, *52*, 517−523.
(27) Furtmüller, P. G.; Arnhold, J.; Jantschko, W.; Pichler, H.; Obinger, C. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 551−557.
(28) Jantschko, W.; Furtmüller, P. G.; Zederbauer, M.; Neugschwandtner, K.; Lehner, I.; Jakopitsch, C.; Arnhold, J.; Obinger, C. *Biochem. Pharmacol.* **2005**, *69*, 1149−1157.
(29) Koppenol, W. H.; Liebman, J. *J. Phys. Chem.* **1984**, *88*, 99−101.
(30) Kettle, A. J.; Winterbourn, C. C. *J. Biol. Chem.* **1994**, *269*, 17146−17151.
(31) van der Walt, B. J.; van Zyl, J. M.; Kriegler, A. *Biochem. Pharmacol.* **1994**, *47*, 1039−1046.
(32) Frost, A. A. *J. Am. Chem. Soc.* **1951**, *73*, 2680−2682.
(33) Zheng, J.; Wang, D.; Thiel, W.; Shaik, S. *J. Am. Chem. Soc.* **2006**, *128*, 13204−13215.

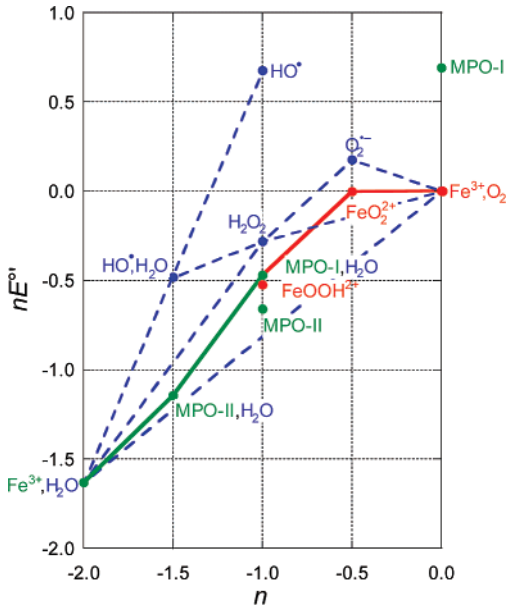


Figure 1. Oxidation state diagram of oxygen with and without cytochrome P450 (partial pressure of dioxygen: 100 kPa, $T = 25$ °C, pH 7). Abscissa: formal charge, n, per oxygen atom and ordinate, n times the electrode potential, $nE^{\circ\prime}$. This quantity is proportional to the Gibbs energy per oxygen atom. The slope of the line joining two points represents an electrode potential; selected electrode potentials are collected in Table 1. Energetics of the reduction of dioxygen to hydrogen peroxide catalyzed by cytochrome P450 is shown in red. The electrode potentials of the couples $P450FeO^{2+}$, $2H^+/P450Fe^{3+}$, H_2O, and $P450FeO^{2+}por^{\bullet+}/P450FeO^{2+}$, in dark green, are assumed to be equivalent to those of the corresponding couples of myeloperoxidase: the slope of the line from myeloperoxidase Fe^{3+}, H_2O at $n = -2$ to myeloperoxidase compound II (MPO-II) at $n = -1$ is +0.97 V and that from MPO-II to myeloperoxidase compound I (MPO-I) at $n = 0$ is +1.35 V. The position of MPO-I is 65 kJ or 0.67 eV above Fe^{3+}, O_2. The point MPO-I, H_2O is found at the intersection of $n = -1$, and the line that joins MPO-I and myeloperoxidase Fe^{3+}, H_2O. The uncatalyzed reduction of dioxygen to water (dashed blue line) takes place via superoxide, hydrogen peroxide, and hydroxyl radical plus water.

P450 appears to be more reactive than MPO-I, and an electrode potential of ca. 1.5 V is necessary at pH 7 for a hydrogen to be abstracted from an aliphatic carbon.[34] However, the rebound step is sufficiently favorable to pull the hydroxylation reaction through. Furthermore, compound I of cytochrome P450 from a thermophilic bacterium appeared to be relatively stable.[17] Thus, a value of +1.35 V is a reasonable estimate for $E^{\circ\prime}$ ($P450FeO^{2+}$-$por^{\bullet+}/P450FeO^{2+}$). Since the conversion of cytochrome $P450FeOOH^{2+}$ to cytochrome $P450FeO^{2+}por^{\bullet+}$, H_2O is fast, cytochrome $P450FeOOH^{2+}$ is unlikely to occupy a location below that of cytochrome $P450FeO^{2+}por^{\bullet+}$, H_2O in the diagram. Given, additionally, that the location of the latter is a lower limit, we estimate an electrode potential $E^{\circ\prime}$(FeO_2^{2+}, H^+/$FeOOH^{2+}$) of 0.93 V at pH7.

A comparison with the oxidation state diagram for the reduction of dioxygen[35] in Figure 1 (blue dashed lines) confirms that cytochrome P450 is eminently suited to activate dioxygen. The binding of dioxygen to iron(II) facilitates the reduction to hydrogen peroxide in that it does not require strong reductants. Hydrogen peroxide is a poor one-electron oxidant, +0.39 V (see Figure 1, line from H_2O_2 to $HO^{\bullet}$, H_2O at $n = -1.5$), and homolysis is energetically unfavorable (Figure 1, H_2O_2 to $HO^{\bullet}$). In general, hydrogen peroxide, although counted among the reactive oxygen species, is very inert. Even if the hydroxyl radical were to be formed, it would not be able to hydroxylate a substrate in a controlled fashion. In contrast, the cytochrome $P450FeOOH^{2+}$ complex is converted isoenergetically to cytochrome $P450FeO^{2+}por^{\bullet+}$, or compound I (Figure 1), which yields, upon reduction, another oxidizing agent, cytochrome $P450FeO^{2+}$, or compound II. Furthermore, cytochromes $P450FeOOH^{2+}$, $P450Fe^{V}O^{3+}$, $P450FeO^{2+}por^{\bullet+}$, and $P450FeO^{2+}$ are likely to be more selective than the hydroxyl radical. Cytochrome $P450FeOOH^{2+}$, which has been postulated as the oxidizing intermediate in certain transformations,[2] is shown here to be as oxidizing as cytochrome $P450FeO^{2+}por^{\bullet+}$, +1.35 V (reaction 10b), while its protonated form would be more oxidizing by ca. 0.05 V. Homolysis of cytochrome $P450FeOOH^{2+}$ to oxidoiron(IV), or compound II, and the hydroxyl radical is energetically uphill by +92 kJ/mol (Figure 1), but, given this barrier and the short half-life of $P450FeOOH^{2+}$, decomposition to compound I and water is likely favored over homolysis.

In a recent ab initio study, values that range from +45 to +72 kJ/mol, depending on the level of theory, were calculated for the homolysis energy of the O−O bond in cytochrome $P450FeOOH^{2+}$,[36] but because these values were calculated for the gas phase, they cannot be compared to the +92 kJ/mol estimated here. Further, the authors of this study used the LACVP basis set to calculate an energy of +162 kJ/mol for the dissociation of HOO^- from $P450FeOOH^{2+}$ in a medium with a dielectric constant of 5.7; it is conceivable that the use of the LAVCP+(d) basis set may reduce this amount by ca. 70 kJ. Correcting this enthalpy by application of a $T\Delta S$ term of ca. 40 kJ/mol yields a Gibbs energy of ca. 50 kJ/mol, in quite good agreement with the 63 kJ/mol value estimated here (J. Harvey, personal communication). In another ab initio study, the energy differences between cytochromes $P450FeO_2^{2+}$ and $P450FeOOH^{2+}$, and between cytochromes $P450FeOOH^{2+}$ and compound I, H_2O, are given as ca. 200 and 40 kJ/mol, respectively.[37] These gas-phase energy differences are quite large and cannot be related to the electrode potentials in this work. Although the uncertainty in ab initio energies may be as high as 20 kJ/mol, errors in the estimations presented here are not likely to be larger than 10 kJ/mol because the values for various thermodynamic parameters are interconnected. For example, when one assumes that the position of MPO-II, H_2O in Figure 1 reflects that of cytochrome P450, then the sum of the association Gibbs energy for cytochrome $P450Fe^{3+}$ and HOO^- and the Gibbs energy for the one-electron reduction of cytochrome $P450FeOOH^{2+}$, relative to NHE, is constant, −203 kJ/mol. Similarly, the sum of $E^{\circ\prime}$ (Fe^{3+}, O_2/FeO_2^{2+}) and $E^{\circ\prime}$ (FeO_2^{2+}, H^+/$FeOOH^{2+}$) should be close to 0.94 V: a lower value would imply a weak association between cytochrome $P450Fe^{3+}$ and hydrogen peroxide, while a higher value would indicate that compound I is insufficiently oxidizing. Clearly, cytochrome P450 strikes a balance between its affinity for hydrogen peroxide and the electrode potential of the compound I/compound II couple. As shown here, the possibilities are not endless but are constrained by the limits set by the energetics of reduction of dioxygen via superoxide and hydrogen peroxide to water (Figure 1, dashed blue lines). Reactions 2, 7, 11, 9b, and 10b must (and do) sum to the reduction of dioxygen to

(34) Koppenol, W. H. *FEBS Lett.* **1990**, *264*, 165−167.
(35) Koppenol, W. H. *Nature* **1976**, *262*, 420−421.
(36) Derat, E.; Kumar, D.; Hirao, H.; Shaik, S. *J. Am. Chem. Soc.* **2006**, *128*, 473−484.
(37) Kamachi, T.; Yoshizawa, K. *J. Am. Chem. Soc.* **2003**, *125*, 4652−4661.

water. Consideration of these imposed limits provides an approach for the calibration of ab initio calculations.

The hydroxylation of a substrate can be represented by reactions 12 and 13

$$P450FeO^{2+}por^{\bullet+} + RH = P450FeOH^{3+} + R^{\bullet} \quad (12)$$

$$P450FeOH^{3+} + R^{\bullet} = P450Fe^{3+} + ROH \quad (13)$$

Green et al.[38] estimated standard bond energies for intermediates in the chloroperoxidase reaction and concluded that the bond enthalpy $D(FeO-H)^{3+}$, ca. 410 kJ/mol, is similar or larger than D(R−H). They argue that, based on a rather long Fe−O distance in cloroperoxidase, oxidoiron(IV) is protonated at pH 7 and that the same may also apply to cytochrome P450; recent ab initio calculations and experimental Mössbauer data appear to confirm that protonation occurs.[39] The second step, reaction 13, can also be analyzed by comparison of D(Fe−OH) with D(C−OH). We assume that, given a pK_a of $P450FeOH^{3+}$ near 7, the energetics of formation of $P450FeO^{2+}$ is similar to that of $P450FeOH^{3+}$ at that pH. Given a $\Delta G_f°$ ($HO^{\bullet}$) of −13 kJ/mol at pH 7, reaction 14

$$P450FeOH^{3+} = P450Fe^{3+} + HO^{\bullet} \quad (14)$$

corresponding to a line from MPO-II to $HO^{\bullet}$ in Figure 1, is uphill by 129 kJ/mol, which is equal to the Gibbs bond energy for $P450Fe-OH^{3+}$. Although the Gibbs energy of reaction 14 is derived from thermodynamic parameters valid at pH 7, it is identical to that under standard conditions. Because it is much smaller than the bond enthalpy of 380 kJ/mol for D(C−OH), obtained by "Bensonizing"[40] a simple alcohol, it does not matter that a Gibbs energy is compared to an enthalpy. Thus, while hydrogen abstraction, reaction 12, is slightly uphill or energetically neutral, the transfer of the hydroxyl group, reaction 13, is very favorable, as has recently been suggested by Groves[41] and as follows from ab initio studies on model compounds.[42]

The thermodynamic parameters and conclusions derived here also apply to other oxygen activating systems. Thus, in the case of a copper monooxygenase, e.g. tyrosinase, the dicopper center must have a certain affinity for hydrogen peroxide that does not imperil its ability to hydroxylate tyrosine. In a Frost diagram, this condition requires the position of the hydrogen peroxide complex to be near that of cytochrome P450. Similar considerations apply to the design of metal complexes that activate dioxygen. First, there is the trivial requirement that the metal site must be both accessible to dioxygen and large enough to accommodate the substrate to be hydroxylated. Furthermore, it is important that the Gibbs energy of formation for the hydroperoxo adduct be somewhat but not too favorable, lest the oxometal state is not oxidizing enough to abstract a hydrogen from the substrate. This requirement implies also that the superoxometal complex must not be too strongly oxidizing, which, in turn, requires that the formation of the superoxo complex is not overly favorable. If the metal-hydroperoxo adduct has a small, but significant, dissociation constant, hydrogen peroxide may dissociate to some extent, as has been observed for cytochrome P450, but, as the concentration of free hydrogen peroxide increases, more of the hydroperoxo-metal complex is formed and enters the catalytic cycle. In addition, good candidate metal complexes will form hydroperoxo complexes that are energetically close to the corresponding oxo complexes. Some complexes of manganese and iron that fulfill these requirements have been reported to hydroxylate substrates, albeit at low temperatures.[41,43] Other candidates should be examined with respect to electrode potentials and dioxygen affinities, which will allow predictions as to their suitability as hydroxylation catalysts.

Acknowledgment. I thank Dr. J. Harvey for clarifying the strengths and weaknesses of ab initio calculations and Drs. R. Kissner and P. L. Bounds for helpful discussions. I thank Dr. G. Merényi for discovering an error in standard state usage that was corrected in proof. The figure was prepared by Dr. P. L. Bounds.

JA071546P

(38) Green, M. T.; Dawson, J. H.; Gray, H. B. *Science* **2004**, *304*, 1653−1656.
(39) Behan, R. K.; Hoffart, L. M.; Stone, K. L.; Krebs, C.; Green, M. T. *J. Am. Chem. Soc.* **2006**, *128*, 11471−11474.
(40) Benson, S. W. *Thermochemical Kinetics*; John Wiley and Sons: New York, 1976.
(41) Groves, J. T. Models and mechanisms of cytochrome P450 action; In *Cytochrome P450: Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Publishers: New York, 2005; pp 1−43.
(42) Shaik, S.; De Visser, S. P.; Altun, A.; Thiel, W. *Chem. Rev.* **2005**, *105*, 2279−2328.
(43) Shan, X.; Que, L., Jr. *J. Inorg. Biochem.* **2006**, *100*, 421−433.